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Effects of Prenatal and Postnatal Exposure of Wi-Fi on Development of Teeth and Changes in Teeth Element Concentration in Rats

Wi-Fi (2.45 GHz) and Teeth Element Concentrations

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Abstract The present study determined the effects of prenatal and postnatal exposure to Wi-Fi (2.45 GHz)-induced electromagnetic radiation (EMR) on tooth and surrounding tissue development as well as the element levels in growing rats. Twenty-four rats and their offspring were equally divided into two separate groups identified as experiment and control. The experiment group was exposed to 2.45 GHz EMR for 2 h/day during the periods of pregnancy (21 days) and lactation (21 days). The offspring of these dams were also exposed to EMR up to decapitation. The control group was exposed to cage stress for 2 h per day using the same protocol established for the experimental group. On the 7th, 14th, and 21st days after birth, 8 male offspring rats from each of the two groups were decapitated, and the jaws were taken for histological and immunohistochemical examination. Caspase-3 (1/50 dilution) was used in the immunohistochemical examination for apoptotic activity. On the last day of the experiment, the rats' incisors were also collected. In samples that were histologically and immunohistochemically examined, there was an increase in apoptosis and caspase-3 in both the control and the Wi-Fi groups during the development of the teeth. However, no significant difference was observed between the two groups in terms of development and apoptotic activity. Results from the elemental analysis showed that iron and strontium concentrations were increased in the Wi-Fi group, whereas boron, copper, and zinc concentrations were decreased. There were no statistically significant differences in calcium, cadmium, potassium, magnesium, sodium, or phosphorus values between the groups. Histological and immunohistochemical examinations between the experimental and control groups showed that exposure to 2.45 GHz EMR for 2 h per day does not interfere with the development of teeth and surrounding tissues. However, there were alterations in the elemental composition of the teeth, especially affecting such oxidative stress-related elements as copper, zinc, and iron, suggesting that short-term exposure to Wi-Fi-induced EMR may cause an imbalance in the oxidative stress condition in the teeth of growing rats.

Keywords Rat tooth development · Developmental effect · Element · MAP, bone morphogenetic proteins · FGF, fibroblast growth factor · ROS, reactive oxygen species · SAR, specific absorption rate · Wi-Fi

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Introduction

In recent times, there has been growing anxiety and speculation about the potential for health risks associated with exposure to electromagnetic radiation (EMR) [1]. A multitude of devices that emit EMR are used in industrial, scientific,

medical, military, and domestic applications. The level of EMR in our environment has increased manifold due to a large-scale expansion of communication networks behind such technologies as mobile phones, base stations, WLAN, 2.45-GHz irradiation-emitting Wi-Fi, etc. [2, 3]. Despite an increasing number of studies into the potential biological effects of EMR and their consequences, data pertaining to its effects on human health are very scarce, and previous studies have reported conflicting results.

Many studies had previously demonstrated the effects of Wi-Fi-induced EMR on brain development, DNA breakage, increased apoptosis, and alteration of homoeostasis in rats [4–6]. Several mechanisms have been proposed to explain the biological effects of EMR on various cellular systems [7, 8], but the exact molecular mechanisms remain unclear. EMR can alter the energy levels and spin orientation of electrons and increase the production of reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, and hydroxyl radicals. Thus, exposure to EMR is associated with enhanced ROS production. Free radicals, presented as ROS, are generated as intermediates in the metabolism and may attack lipids, proteins, and nucleic acids [2, 9]. Tissue development and repair-related second messengers and signaling pathways, such as bone morphogenetic proteins (BMPs), fibroblast growth factor (FGF), and Wnt, are in interaction with ROS [10–12]. The earliest interactions regulate tooth initiation, and the last govern the secretiom and mineralization of dentin and enamel as well as root development [13]. FGF and Wnt are responsible for its maintenance, and BMP is responsible for its patterning. It is important to note that these pathways, in turn, depend on each other [14]. In addition, some of the literature mentioned that EMR directly interacts with cell membranes and altered trace element concentrations in plasma and cerebrospinal fluid [15]. Moreover, there are a limited number of manuscripts on the effects of EMR on the teeth. These determined alterations in element concentrations [16, 17]. Hence, the Wi-Fi exposure-induced ROS may affect the development and metal composition in the teeth of developing rats, and the subject merits further clarification.

Tooth morphogenesis and the subsequent differentiation of the epithelial ameloblasts and the ectomesenchymal odontoblasts are followed by the formation and mineralization of the enamel and dentin matrices, respectively. Epithelial-mesenchymal interactions inducing and governing the development of the teeth (and of many other organs) involve the sequential expression of hierarchic genes and proteins, such as growth factors, homeobox-containing transcription factors, receptors, and matrix molecules [13, 18]. In the literature, the interactions between dental hard tissue formation and malnutrition, radiation, chemicals, and drugs are very well-established [19–21]. It is noteworthy that, once formed, the dental hard tissues are not remodeled; hence, any major disturbances to the function of the dental cells will be permanent [22].

Trace elements play an important and complex role in human and animal metabolisms [23]. Laboratory animal teeth have been used as indicators of exposure to several trace elements. The trace elements in the teeth have been examined for a number of reasons; for example, some dental health studies have correlated trace element contents with the presence of dental caries [23]. The importance of measuring trace elements in the teeth was also underscored by their importance as bioindicators, connecting the deposited chemical elements in the tooth to the environment and/or dietary habits, and with the promotion or inhibition of tooth cavities [24–26].

To our knowledge, there is no report on Wi-Fi-induced alterations of trace element levels in the teeth of developing rats, humans, or other animals. Moreover, we could not find any reports on the effects of EMR on tooth development. Our hypothesis was based on its harmful effect on the cell membrane and alterations to the permeability of the membrane against some elements. It is well reported that the destruction of cell membranes leads to alterations in elemental concentration. EMR-related changes in oxidative stress levels due to adverse effects in the signaling pathways could have a detrimental effect on tooth development. Hence, we aimed to determine the effects of Wi-Fi (2.45 GHz)-induced EMR on the development of rat teeth and some trace element contents of rat incisors.

Materials and Methods

Animals

This study was planned and organized as completely double blind. Approval for the study was obtained from Medical Faculty Experimentation Ethics Committee of Suleyman Demirel University (approval number 26.10.2010-01). The care and use of the animals were conducted in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Suleyman Demirel University. Female Wistar Albino rats (n=24) of 10–12 weeks weighing between 180–200 g obtained from the Suleyman Demirel University Animal Experiments Laboratory were used in the current experiment. The rats were maintained individually in stainless steel cages with optimized living conditions and fed a commercial diet.

To obtain newborns, animals were mated in the proportion of one male to three females. Once the presence of sperm was detected, the female was considered pregnant, and this day was considered to be day 0 of the pregnancy. Twenty-four rats were equally divided into two separate groups as experiment and control. The experiment group was exposed to 2.45 GHz EMR for 2 h/day during the periods of pregnancy (21 days)

and lactation (21 days). Offspring (n=48) of these dams were also exposed to EMR up to decapitation. The control group was exposed to cage stress 2 h in a day using the same protocol established for the experimental group. EMR was applied daily between 11:30 and 13:30.

Exposure System and Design

For experimental exposure, a SET ELECO (Set Electronic Co./Istanbul) radio frequency (RF) generator providing a 2.45-GHz RF emission, pulsed with 217 Hz, was used with a half-wave dipole antenna system. This device is capable of producing 0.1 V/m to 45.5 V/m electric field densities. The overall system performance of the exposure device was tested and verified at the Laboratory of the Department of Electronics and Communication Engineering (Süleyman Demirel University, Isparta, Turkey). The exposure design and methodology were adapted from a similar study [27]. The exposure system was deployed in a specific room that contained plastic furniture such as tables and chairs in order to protect the animals from potential radiation and reflected emissions. The walls of the room were completely covered with chromium–nickel sheets to protect the animals from exposure to radiation from external sources. All six rats per group were exposed at the same time in the exposure system. This device is organized with a specialized cylindrical PVC restrainer, which provides appropriate exposure conditions, and to accommodate the physical size of one rat (for pups/offspring length 15/5 cm, diameter 5/3 cm). The noses of the rats were positioned in close contact to the monopole antenna, and the tube was ventilated from the head to tail in order to decrease the stress on the rats while in the tube. Repetition time, frequency, and amplitude of the RF energy spectrum were observed and verified by means of a satellite level meter (PROMAX, MC-877C, Barcelona/Spain). All of the reflection and exposure measurements were carried out by means of a Portable RF Survey System (HOLADAY, HI-4417, Minnesota, USA) equipped with its standard probe. The electromagnetic dosimetry is calculated by using measured electric field density (V/m) and digital anatomical models based on the FDTD numerical code. Consequently, a specific absorption rate (SAR) value was predicted for the same condition, orientation, and antenna power by using this method as 0.009±0.002 W/kg per head. The rats of the sham exposure group were placed in the restrainer individually with the RF source switched off during experiments. All exposure systems were kept in a Faraday cage. All of the exposure procedures were carried out in this cage, which had a shielding effectiveness of 100 dB. As well, the exposure of each group was not permitted to affect the other groups. To verify this result, data were taken continuously by means of an RF measurement apparatus within the experiment room described above.

Teeth and Tissue Collection

The animals were anesthetized by intraperitoneal injection of a combination of ketamine (25 mg/kg) and xylazine (10 mg/kg) and sacrificed. Eight male offsprings were randomly selected from each of the dams on the 7th, 14th, and 21st days, and histopathological analyses were performed on the molar region. Also, the rats' incisors were collected for trace elemental analysis at 21 days of life.

Histopathological and Immunohistochemical Examination

Jaw samples of the rats were fixed in 10 % buffered formalin. For the immunostaining and microscopy analyses, samples were decalcified in 4 % EDTA at neutral pH to allow for better discrimination. As per routine procedure, tissues were blocked in paraffin and cut to 5-μm thickness in sagittal sections. The tissue sections were stained with hematoxylin-eosin (H&E) and examined microscopically. Afterward, the jaw samples were immunostained with caspase-3 (rabbit polyclonal, Cat. no. 250573, Abbiotec-San Diego, USA) according to the manufacturer's instructions. In this study, the avidin-biotin complex peroxidase (ABCP) method was used for immunohistochemistry. Paraffin blocks were sectioned at 5 μm for immunohistochemical examination, and sections were attached to glass slides coated with poly-L-lysine. The slides were dried overnight at 37 °C in order to optimize adhesion. Sections were deparaffinized by means of xylene, and tissues were rehydrated in sequentially graduated ethyl alcohol. Slides were incubated in hydrogen peroxide in methanol for 10 min to reduce nonspecific background staining due to endogenous peroxidase. The sections were washed twice in phosphate buffer solution (PBS). The tissues were then boiled in a 1:100 citrate buffer solution for 10 min and cooled for 20 min. The cooled tissues were washed four times in PBS prior to application of blocking serum for 5 min. Primary antibody was applied, and then, tissues were incubated for 30 min at room temperature. They were rinsed four times in PBS, given an application of biotinylatedantipolyvalent antibody, and incubated for 10 min at room temperature. After being washed three times in PBS, streptavidin peroxidase was applied, and the samples were incubated for 10 min at room temperature, and then rinsed four times in PBS. Tissues were further incubated for 20 min at room temperature in a solution of 3,30-diaminobenzidine (DAB) chromogen. After being washed in PBS, tissues were counterstained with Mayer's hematoxylin, washed in water, and coverslips were applied with mounting media. For negative control, primary antibody was not added to the sections.

To evaluate the severity of the immunohistochemical reaction of tumor cells with markers, semiquantitative analysis was performed using an arbitrary visual scale with a grading score ranging from (−) to (+++) as follows: (−)=negative, (+

)=weak staining, (++)=mild staining, (+++)=strong staining. In order to evaluate the percentage of immunopositive cells, 100 cells calculated in 10 different microscopic high-powered fields of each slide were examined under the 40× objective of a trinocular microscope (Nikon E600) and microphotography apparatus. The count of positive cells one high-power field for each marker was noted and compared with control groups.

Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) Elemental Analysis

The determination of calcium (Ca), iron (Fe), zinc (Zn), boron (B), copper (Cu), strontium (Sr), cadmium (Cd), potassium (K), magnesium (Mg), sodium (Na), and phosphorus (P) was performed on a model Perkin Elmer Optima 5300 DV ICP-OES under optimized measurement condition.

For the ICP analysis, each tooth was individually decoronated, the root discarded, and the pulp removed. Samples from the teeth were then decomposed in a microwave oven (Milestone ETHOS One, Suarlée, Belgium) with 5-mL trace-pure HNO_3 (SCP Science, NY) and 1 mL H_2O_2, and diluted to 15 mL using double-DI water. These tooth solutions were quantitatively analyzed for the elements in an ICP-OES.

The ICP-OES parameters used were the following: nebulizer flow, 0.80 L/min; power, 1450 W; peristaltic pump rate, 1.5 mL/min; flush time, 30 s; delay time, 20 s; read time, 10 s; wash time, 60 s; and each sample was read in triplicate.

Statistical Analysis

All results are expressed as means±standard deviation. p Values of less than 0.05 were regarded as significant. Significant values were assessed by means of independent sample t test. Data was analyzed using the SPSS statistical program (version 17.0 software, SPSS Inc. Chicago, IL, USA).

Results

Body Weight Changes and Macroscopic Findings

The body weights of 7-, 14-, and 21-day-old rats are shown in Table 1. There is no statistically significant difference between the 2.45-GHz EMR group and the control group ($p>0.05$).

We did not observe any cases of tooth agenesis in either the experimental group or the controls, and the developmental chronology was consistent from the tooth germ to the final phase of the crown.

Table 1 The weights (g) of 7-, 14-, and 21-day-old rats of control and Wi-Fi groups

Age (postnatal day)	Control	Wi-Fi
7	11.35±1.45	11.35±0.74
14	18.49±2.66	16.38±2.14
21	25.04±4.56	25.12±3.76

Histopathological Findings

First and second molars of rats were observed far below the epithelial layer on the 7th day postpartum (Fig. 1a, b), while they were very close to the epithelial layer on the 14th day postpartum (Fig. 2a, b). All three cusps of the first and second molars had penetrated the oral mucosa. Root development was ongoing in the second and first molars with the apices open on the 21st day postpartum (Fig. 3a, b). There were no pathological changes to the enamel and dentin layer. There were no abnormalities in the layers of the teeth, and no differences between the groups were observed.

Immunohistochemical Findings

In the immunohistochemical staining for caspase-3, there were mild caspase-3 reactions detected in the oral mucosa epithelium in both groups. Similar reactions were determined in odontoblasts and ameloblasts in both groups (Fig. 4a, b). There were no differences in caspase-3 expression between 7th, 14th, and 21st days.

Element Results

It was determined that Ca, Cd, K, Mg, Na, and P values in teeth are not statistically significant ($p>0.05$) (Table 2). In addition, iron and strontium concentrations are significantly ($p<0.05$) higher in the Wi-Fi group than those in the control group (Fig. 5). However, boron, copper, and zinc concentrations are significantly ($p<0.01$) higher in the control group than those in the Wi-Fi group (Figs. 6 and 7).

Discussion

Nowadays, wireless devices are commonly used in all areas of life. Modern wireless devices operate on frequency ranges (2400–2500 Hz) that are higher than those used by cell phones and typically entail longer exposure times and wider areas of exposure. Devices that use such wireless connection technology are primarily used in proximity to the abdominal region, where the fetus is located and may cause harmful effects to its organ development and differentiation.

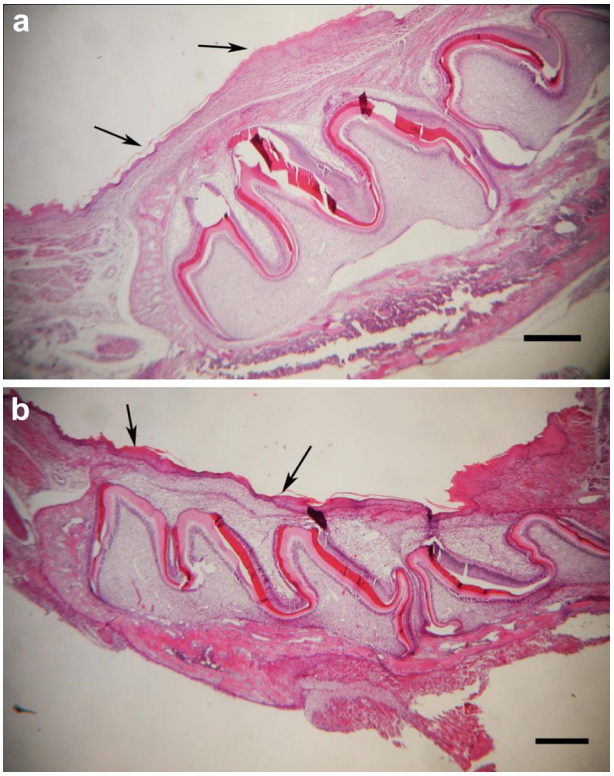

Fig. 1 **a** Tooth development of 7-day-old rat in control group. The teeth have not yet erupted, and they were located below the epithelium (*arrows*). H&E, *bar*=400 μm. **b** Tooth development of 7-day-old rat in Wi-Fi group. The teeth have not yet erupted, and they were located below the epithelium (*arrows*). H&E, *bar*=400 μm

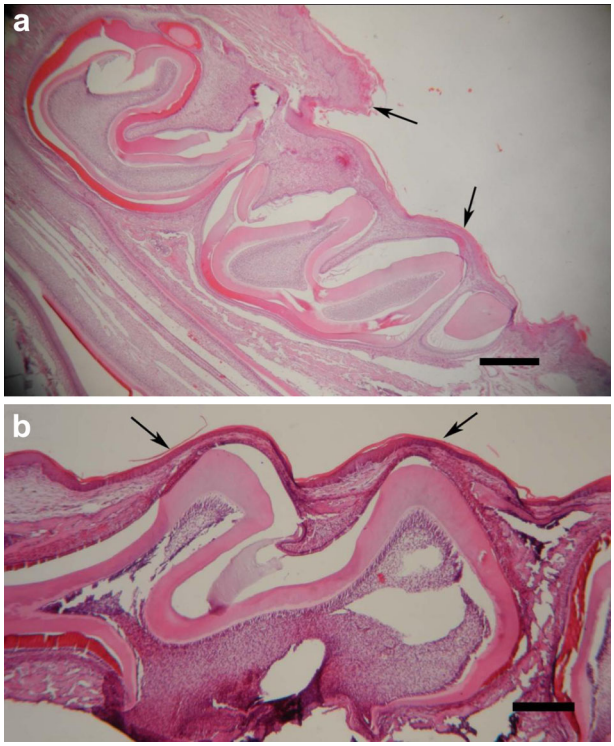

Fig. 2 **a** Tooth development of 14-day-old rat in control group. The teeth have not yet erupted, but they were located just below the epithelium (*arrows*). H&E, *bar*=400 μm. **b** Tooth development of 14-day-old rat in Wi-Fi group. The teeth have not yet erupted, but they were located just below the epithelium (*arrows*). H&E, *bar*=200 μm

To the best of our knowledge, the present study is the first to investigate the effects of Wi-Fi (2.45 GHz)-induced EMR on the development of teeth and the surrounding tissues as well as on the levels of elements in rat teeth; therefore, the results of this study are not directly comparable to those of previous studies. However, considering the differences between the methodology used in the present study and those methodologies used in previous studies, such as EMR exposure time and frequency, analytical methods, and the source of tissue, some useful comparisons can be made with respect to the control group.

Increase in body weight is associated with the general development of the body. Our results showed no differences in body weight. Histological analysis revealed no differences between groups. These results indicate that 2.45 GHz EMR does not affect the development of the teeth and their surrounding tissues in rats.

Apoptosis is the process of programmed cell death, which is mediated by specific proteinases, namely caspases. There are two major pathways of apoptosis, related to caspase-8 and caspase-9 activation, and both of these pathways induce caspase-3 activation [28]. It has been suggested that EMR causes apoptosis in several tissues through an increased caspase-3 activity. Palumbo et al. [29] reported that 900-MHz GSM radiation increased caspase-3 activity in human lymphocytes. Exposure to EMR at 2.45 GHz has been reported to cause an increase in caspase activity, which is dependent on apoptosis, in the reproductive system [30]. However, Agustino et al. [31] also suggested that EMR at the same frequency (2.45 GHz) alters the levels of cellular stress in rat thyroid glands without inducing apoptosis. In the present study, although caspase-3 activity was increased in some areas of the teeth and surrounding tissues, we believe that this observation is associated with a normal process of growth and development.

Dental tissues consist of an organic matrix and inorganic constituents. Studies have showed that the teeth with an accurate macrostructure and microstructure and an adequate degree of mineralization are much more resistant to cariogenic factors [32]. The teeth, both in humans and in animals, are often regarded as biological indicators of environmental hazards. The trace elements found in the teeth are essential to humans, and their appropriate concentrations and ratios in dental tissues are associated with normal biological function and development. Therefore, the effects of 2.45 GHz-induced EMR on element levels in rat teeth were investigated. We found no statistically significant differences in calcium, potassium, magnesium, sodium, phosphorus, or cadmium levels

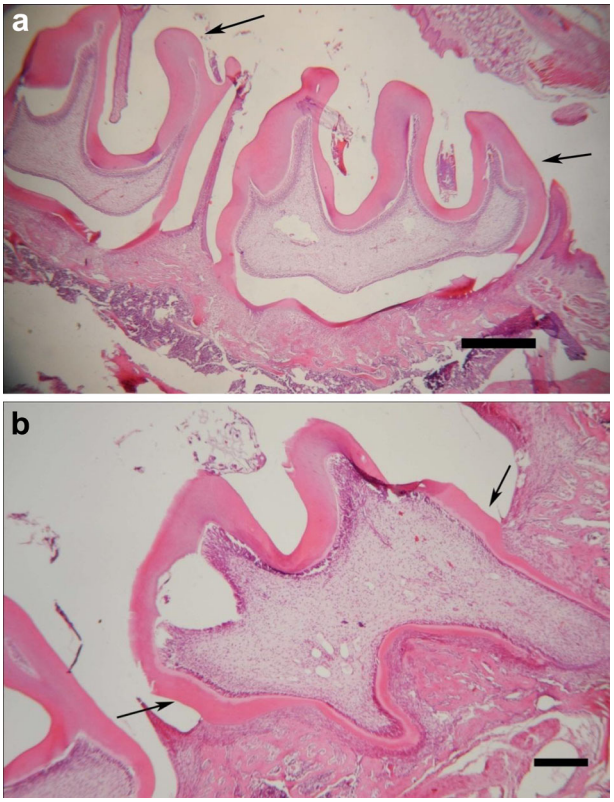

Fig. 3 **a** Tooth development of 21-day-old rat in control group. The teeth have fully erupted (*arrows*), and tooth structures are in normal view. H&E, *bar*=200 μm. **b** Tooth development of 21-day-old rat in Wi-Fi group. The teeth have fully erupted (*arrows*), and tooth structures are in normal view. H&E, *bar*=200 μm

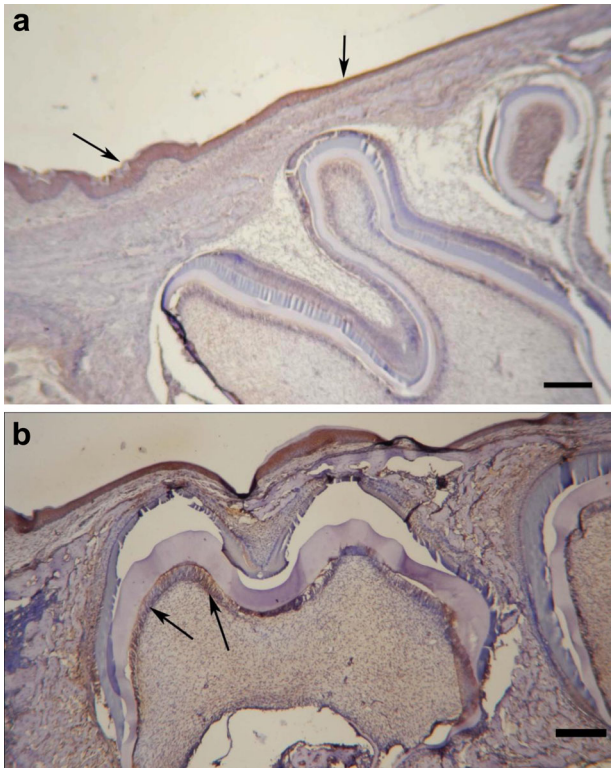

Fig. 4 **a** Positive caspase-3 reaction in epithelium (*arrow*) in 7-day-old rat in control group, streptoavidinbiotin peroxidase method, *bar*= 200 μm. **b** Positive caspase-3 reaction in odontoblasts (*arrow*) in 14-day-old rat in Wi-Fi group, streptoavidin biotin peroxidase method, *bar*= 200 μm

between the control and Wi-Fi groups. Results from the elemental analysis also showed that the iron and strontium concentrations increased in the Wi-Fi group, whereas boron, copper, and zinc concentrations decreased.

Ca, Mg, P, K, and Na are essential for living organisms; these macroelements are present in the teeth, where they form the basic structural components of hydroxyapatite. Decreased concentrations of these elements in dental hard tissue may cause deleterious effects, such as susceptibility to caries. In our study, we observed similar levels of these elements in the control and Wi-Fi groups. These findings are consistent with the results of a previous study by Adıgüzel et al. [16] that showed that EMR (900 MHz) from mobile phones did not affect the Ca and P levels in rat dental hard tissues. However, in the same study, they found reduced concentrations of Mg in the Wi-Fi groups, which is in contrast to the results obtained in the present study.

A positive correlation has been shown between the Sr levels in enamel apatite and tooth hardness and acid resistance in the teeth [33]. However, it has also been reported that there is no association between the Sr content of the teeth and the incidence of dental caries [34]. Owing to the chemical similarity to Ca ions, Sr ions can easily incorporate into the calcified matrix of hard tissues. Kaya et al. [17] were able to relate the differences observed between experimental and control groups with changes in the chemical bonding of the trace elements. It was also shown that oxidative stress

Table 2 Effects of Wi-Fi (2.45 GHz) EMR exposure on calcium, cadmium, potassium, magnesium, sodium, and phosphorus levels in incisor samples of 21-day-old rats (n=8, mean±SD) (p>0,05)

Parameters	Control	Wi-Fi
Calcium (g/kg teeth)	203.1±10.1	204.6±9.5
Cadmium (mg/kg teeth)	0.3±0.03	0.2±0.03
Potassium (g/kg teeth)	3.1±0.3	3±0.2
Magnesium (g/kg teeth)	8.5±0.6	8.4±0.3
Sodium (g/kg teeth)	6.9±0.3	7.8±0.6
Phosphorus (g/kg teeth)	1.1±0.08	1.1±0.03
Iron (mg/kg teeth)	85.4±11.1	153.8±19.2
Strontium (mg/kg teeth)	107.6±9.6	155.4±8.9
Boron (mg/kg teeth)	10.9±1.1	2.5±0.4
Copper (mg/kg teeth)	15.5±1.5	5.3±1.2
Zinc (mg/kg teeth)	64.2±1.7	46±3.3

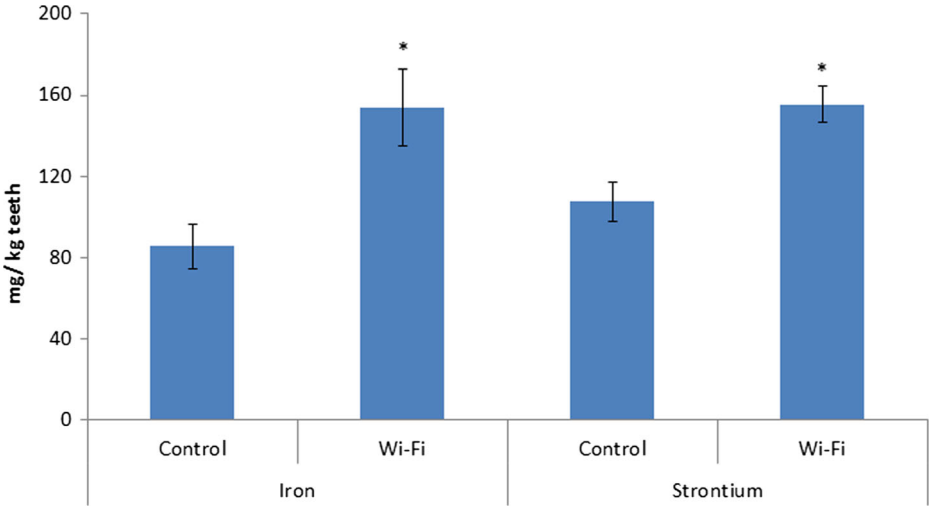

Fig. 5 The levels of iron and strontium in control and Wi-Fi group of Rats.*$p<0.01$ and versus control

associated with EMR disrupts ion transport [35]. It should be noted that this difference could be related to a genetic trait.

Zn and Cu are important trace elements in the human body. Zn has an important role in protein synthesis and is a cofactor for many enzymes that regulate gene transcription, growth factor metabolism, hormone levels, and cell growth. Therefore, Zn plays an important role in the formation of mineralized tissues and in metabolism. Animal studies suggest that the Zn concentrations in dental hard tissue reflects metal absorption [26]. It has been suggested that these elements provide protection against oxidative stress by functioning as cofactors for antioxidant enzymes such as superoxide dismutase [36].

Fe is also a trace element, and it has been shown to be associated with oxidative stress; a positive correlation between Fe and ROS levels has also been reported. Several reports have indicated that EMR affects ROS levels in cells via Fenton reactions of metals such as Zn, Cu, and Fe [37, 38]. Zn and Cu act as antioxidants by decreasing ROS levels [37]. Fe plays a key role in the important Fenton reactions of the cell, which are involved in the generation of free radicals by chemicals that are present in vivo, such as Fe and H_2O_2. Increased Fe levels trigger OH^- formation, which is more harmful to cell physiology in relation to lipid peroxidation [39]. In the present study, B, which has beneficial effects on the metabolism of many biological compounds and on tissue function [40], was found at lower levels in the Wi-Fi group than in the control group. It has recently been suggested that B is effective in the prevention of oxidative stress and DNA damage in rats [41]. We found significantly lower levels of Zn and Cu, which also play important roles in antioxidant metabolism, and high levels of Fe, which were shown to have an effect on oxidative stress in the Wi-Fi group; these results indicate that 2.45 GHz EMR causes changes in the elemental content of dental hard tissue via oxidative stress. We believe that the observed increase in Fe levels is due to Fe oxidation in the tissues via Fenton reactions [39]. We did not observe any differences in the histopathological examinations of the Wi-Fi and control groups, which suggests that prenatal and postnatal exposure to Wi-Fi-induced EMR for 2 h a day does not

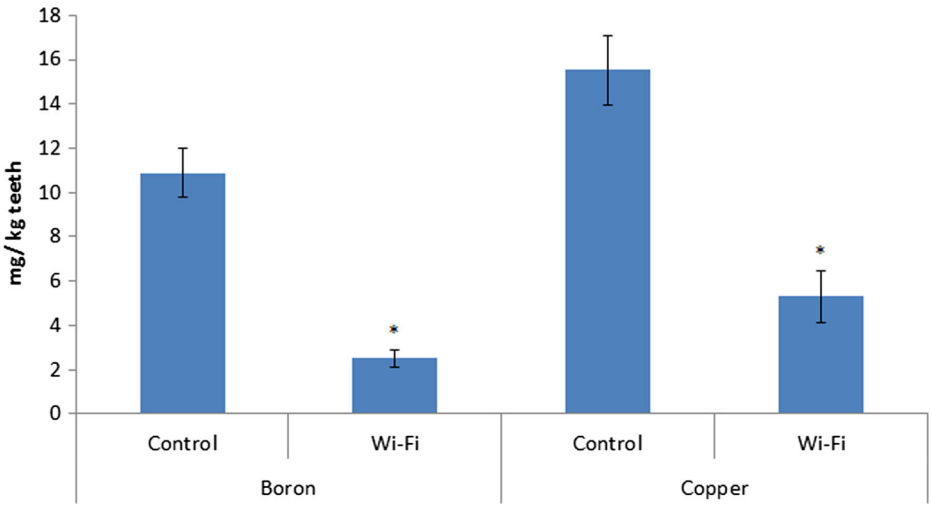

Fig. 6 The changes of teeth boron and copper levels in control and Wi-Fi groups of rats.*$p<0.01$ and versus control

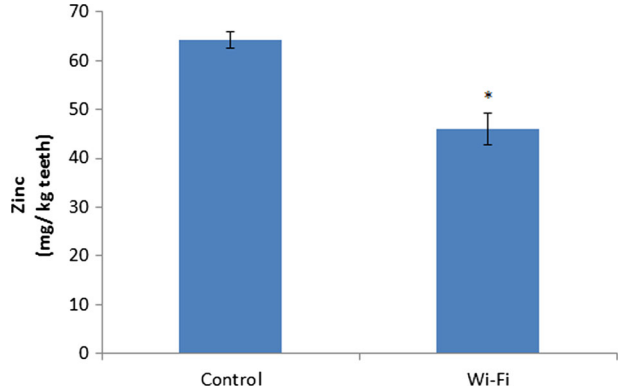

Fig. 7 Teeth zinc levels in control and Wi-Fi groups of rats.*$p<0.01$ and versus control

interfere with the development of the teeth and their surrounding tissues. However, EMR-induced effects are not instantaneous, unlike those induced by chemical and environmental agents; they may arise due to the accumulation of cumulative interactions. Furthermore, the animals in our study were exposed to Wi-Fi-induced EMR for approximately 40 days, depending on the tooth development stages in the rats. Considering that this period is equivalent to approximately 10 years in humans, it is clear that the exposure period of our study is of too short a duration to draw conclusions as to the effects of Wi-Fi exposure over a lifetime.

Furthermore, this study determined the content and distribution of trace elements in rat teeth that were exposed to Wi-Fi. It was observed that the elemental content of the crowns of the teeth of the rats differed significantly between the two groups. Differences in the levels of elements in the teeth, especially those of oxidative stress-related elements, suggest that short-term exposure to Wi-Fi-induced EMR causes oxidative injury to the teeth of growing rats. However, the possibility that these differences are related to genetic traits should be considered.

Considering the increased use of wireless networks and the increased duration of its use, we suggest that studies involving a larger number of subjects and longer periods of exposure to Wi-Fi should be undertaken to further analyze the effects of Wi-Fi on the development of the teeth and their surrounding tissues.

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Authors' Roles ZZÇ, ZK and MN formulated the present hypothesis and MN was also responsible for writing the report. ÖÖ was responsible for pathological analyses. ZZÇ and ZK were repsonsible for experimental procedure of the study. ZK made critical revisions to the manuscript.

Conflict of Interest None of the authors have any conflicts to disclose. All authors have approved the final manuscript.

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